Claims

What is claim d is:

1. A method for determining the mode of action of an antimicrobial compound, comprising:

(a) detecting hybridization complexes formed by contacting at least one nucleic acid sample, obtained by culturing cells of a bacterium in the presence of at least one sub-inhibitory amount of an antimicrobial compound having an unknown mode of action, with a plurality of nucleic acid sequences corresponding to genes of the bacterial cells, wherein the presence, absence or change in the amount of the hybridization complexes detected, compared with hybridization complexes formed between the plurality of nucleic acid sequences and a second nucleic acid sample obtained from the bacterial cells cultured in the absence or presence of a standard compound having a known mode of action, is indicative of the similarity or dissimilarity of the mode of actions of the antimicrobial compound and the standard compound; and

(b) assigning a mode of action for the antimicrobial compound based on the similarity or dissimilarity of values assigned to the hybridization complexes detected in (a) based on the relative amount of hybridization to a second set of hybridization values assigned to the hybridization complexes formed from the second nucleic acid sample.

2. The method of claim 1, wherein the step in (b) comprises subjecting the set of values to a program for analysis.

3. The method of claim 2, wherein the program for analysis comprises a computer algorithm.

4. The method of claim 1, wherein the bacterium is *Bacillus subtilis.*

5. The method of claim 1, wherein the bacterium is *Escherichia coli.*

6. The method of claim 1, wherein the bacterium is a *Staphyloccous* sp.

7. The method of claim 6, wherein the bacterium is *Staphylococcus aureus.*

8. The method of claim 6, wherein the bacterium is *Staphylococcus epidermidis.*

9. The method of claim 1, wherein the bacterium is selected from the group consisting of *Shigella dysenteriae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Burkholderia cepacia, Acinetobacter boumanii, Neisseria gonorrhoeae, Haemophilus influenzae,* and *Stenotrophomas maltophilia.*

10. The method of claim 1, wherein the bacterium is selected from the group consisting of *Streptococcus pneumoniae, Enterococcus faecalis, Enterococcus faecium,* and *Mycobacterium tuberculosis.*

11. The method of claim 1, wherein the antimicrobial compound is a member of the class of antimicrobial compounds that inhibit cell wall synthesis, interfere with the cell membrane, inhibit protein synthesis, inhibit topoisomerase activity, inhibit RNA synthesis, or is a competitive inhibitor.

12. The method of claim 1, wherein the standard compound is a member of the class of antimicrobial compounds that inhibit cell wall synthesis.

13. The method of claim 12, wherein the standard compound is a penicillin, cephalosporin, or bacitracin.

14. The method of claim 12, wherein the standard compound is cephalothin or vancomycin.

15. The method of claim 1, wherein the standard compound is a member of the class of antimicrobial compounds that interfere with the cell membrane.

16. The method of claim 15, wherein the standard compound is a polymyxin or gramicidin.

17. The method of claim 1, wherein the standard compound is a member of the class of antimicrobial compounds that inhibit protein synthesis.

18. The method of claim 17, wherein the standard compound is selected from the group consisting of tetracycline, chloramphenicol, aminoglycoside, macrolide, and gramicidin.

19. The method of claim 1, wherein the standard compound is a member of the class of antimicrobial compounds that inhibit topoisomerase activity.

20. The method of claim 19, wherein the standard compound is selected from the group consisting of novobiocin, nalidixic acid, ciprofloxacin, and norfloxacin.

21. The method of claim 1, wherein the standard compound is a member of the class of antimicrobial compounds that inhibit RNA synthesis.

22. The method of claim 21, wherein the antimicrobial compound is a rifamycin or nalidixic acid.

23. The method of claim 1, wherein the standard compound is a competitive inhibitor.

24. The method of claim 23, wherein the standard compound is trimethoprim.

25. The method of claim 1, wherein the plurality of sequences are obtained from the same organism as the bacterium.

26. The method of claim 1, wherein the plurality of sequences are obtained from an organism different from the bacterium.

27. The method of claim 1, wherein the plurality of sequences are obtained from *Bacillus subtilis*.

28. The method of claim 25, wherein the plurality of sequences correspond to less than about 75% of the genome of the bacterial cells.

29. The method of claim 25, wherein the plurality of sequences correspond to less than about 50% of the genome of the bacterial cells.

30. The method of claim 25, wherein the plurality of sequences correspond to less than about 25% of the genome of the bacterial cells.

31. The method of claim 25, wherein the plurality of sequences correspond to less than about 10% of the genome of the bacterial cells.

32. The method of claim 25, wherein the plurality of sequences correspond to less than about 5% of the genome of the bacterial cells.

33. The method of claim 1, wherein the plurality of sequences correspond to less than about 2% of the genome of the bacterial cells.

34. The method of claim 1, wherein the plurality of nucleic acid sequences is contained on a substrate.

35. The method of claim 34, wherein the substrate is a microarray, macroarray, Southern blot, zoo blot, slot blot, dot blot, or Northern blot.

36. The method of claim 1, further comprising:

(c) identifying from the plurality of nucleic acid sequences at least one sequence, or a homolog thereof, from the nucleic acid sample obtained from the bacterial cells cultivated in the presence of the antimicrobial compound that has a detected expression level that is significantly different from the nucleic acid sample obtained from bacterial cells cultivated in the absence of the antimicrobial compound.

37. The method of claim 36, wherein the difference in the detected expression level is at least about 10% or greater.

38. The method of claim 36, wherein the difference in the detected expression level is at least about 20% or greater.

39. The method of claim 36, wherein the difference in the detected expression level is at least about 50% or greater.

40. The method of claim 36, wherein the difference in the detected expression level is at least about 75% or greater.

41. The method of claim 36, wherein the difference in the detected expression level is at least about 100% or greater.

42. The method of claim 36, further comprising:

(d) isolating a sequence identified in (c) or a homolog thereof.

43. The method of claim 42, wherein the sequence is a marker of the antimicrobial compound.

44. The method of claim 1, wherein the plurality of nucleic acid sequences is selected from the group of genes of Tables 4-21 or fragments thereof.

45. The method of claim 1, wherein the plurality of nucleic acid sequences is a marker gene for the mode of action of topoisomerase activity inhibition selected from the group of genes in Tables 4, 5, 6 or 7, or fragments thereof.

46. The method of claim 1, wherein the plurality of nucleic acid sequences includes *yerQ* or a fragment thereof.

47. The method of claim 1, wherein the plurality of nucleic acid sequences is a marker for the mode of action of cell wall inhibitors selected from the group of genes of Tables 8, 9, and 10, or fragments thereof.

48. The method of claim 1, wherein the plurality of nucleic acid sequences is a marker for the mode of action of protein synthesis inhibitors selected from the group of genes of Tables 11-20.

49. The method of claim 1, wherein the plurality of nucleic acid sequences is a marker gene for the mode of action of RNA synthesis inhibition selected from the group of genes in Table 21, or fragments thereof.

50. The method of claim 1, wherein the plurality of nucleic acid sequences is obtained from *Staphylococcus aureus*.

51. The method of claim 50, wherein the plurality of nucleic acid sequences includes SA0681 or a fragment thereof, which is a marker gene for the mode of action of topoisomerase activity inhibition.

52. The method of claim 50, wherein the plurality of nucleic acid sequences includes SP1714 or a fragment thereof, which is a marker gene for the mode of action of topoisomerase activity inhibition.

53. The method of claim 1, wherein the plurality of nucleic acid sequences is obtained from *Streptococcus pneumoniae*.

54. The method of claim 53, wherein the plurality of nucleic acid sequences includes SP1045 or a fragment thereof, which is a marker gene for the mode of action of topoisomerase activity inhibition.

55. An isolated nucleic acid obtained by the method of claim 42, which is selected from the group consisting of the genes of Tables 4-23.

56. A substrate comprising the plurality of nucleic acid sequences selected from the group of genes of Tables 4-21 or fragments thereof.

57. The substrate of claim 56, wherein the plurality of nucleic acid sequences is a marker for the mode of action of topoisomerase activity inhibition selected from the group of genes of Tables 4, 5, 6 or 7, or fragments thereof.

58. The substrate of claim 56, wherein the plurality of nucleic acid sequences includes *yerQ* or a fragment thereof.

59. The substrate of claim 56, wherein the plurality of nucleic acid sequences is a marker for the mode of action of cell wall inhibitors selected from the group genes of Tables 8, 9, and 10, or fragments thereof.

60. The substrate of claim 56, wherein the plurality of nucleic acid sequences is a marker for the mode of action of protein synthesis inhibitors selected from the group of Tables 11-20.

61. The substrate of claim 56, wherein the plurality of nucleic acid sequences is a marker for the mode of action of RNA synthesis inhibitors selected from the group of Table 21.

62. The substrate of claim 56, wherein the plurality of nucleic acid sequences is obtained from *Staphylococcus aureus*.

63. The substrate of claim 62, wherein the plurality of nucleic acid sequences includes SA0681, or a fragment thereof, which is a marker for the mode of action of topoisomerase activity inhibition.

64. The substrate of claim 62, wherein the plurality of nucleic acid sequences includes SA1714, or a fragment thereof, which is a marker for the mode of action of topoisomerase activity inhibition.

65. The substrate of claim 56, wherein the plurality of nucleic acid sequences is obtained from *Streptococcus pneumoniae*.

66. The substrate of claim 65, wherein the plurality of nucleic acid sequences includes SP1045, or a fragment thereof, which is a marker for the mode of action of topoisomerase activity inhibition.

67. A computer readable medium, comprising one or more nucleic acid sequences selected from the group of genes in Tables 4-21 or fragments thereof.

68. A computer-based system for analyzing hybridization complexes formed by contacting at least one nucleic acid sample, obtained by culturing cells of a bacterium in the presence of at least one sub-inhibitory amount of an antimicrobial compound having an unknown mode of action, with a plurality of nucleic acid sequences corresponding to genes of the bacterial cells, wherein the presence, absence or change in the amount of the hybridization complexes detected, compared with hybridization complexes formed between the plurality of nucleic acid sequences and a second nucleic acid sample obtained from the bacterial cells cultured in the absence or presence of a standard compound having a known mode of action, is indicative of the similarity or dissimilarity of the mode of actions of the antimicrobial compound and the standard compound, said computer-based system comprising the following elements:

(a) a data storage means;

(b) a search means; and

(c) a retrieval means.

69. A method for evaluating a compound for antimicrobial activity, comprising testing the compound for inhibition, interaction, or interference with the normal expression or activity of the corresponding bacterial gene of claim 42.

70. The method of claim 69, further comprising:

(e) testing for essential activity of the expression of the bacterial gene in (d).

71. The method of claim 70, wherein the bacterial gene in (d) is prepared as a knockout.

72. The method of claim 70, wherein the bacterial gene in (d) is repressed.

73. The method of claim 70, wherein the bacterial gene in (d) is induced.

74. The method of claim 70, wherein the bacterial gene in (d) is mutagenized.

75. A method for screening for an antimicrobial compound having a mode of action of interest, comprising:

(a) treating bacterial cells with a test compound, wherein the bacterial cells comprise a responsive promoter linked to a reporter gene; and

(b) detecting the expression of the reporter gene;

wherein the responsive promoter is a promoter which is induced in a cell which is treated by an antimicrobial compound of a first class of antimicrobial compounds, but not by an antimicrobial compound of a second class of antimicrobial compounds, and

wherein the presence, absence or change in the amount of the expression of the reporter gene is indicative of the similarity or dissimilarity of the mode of actions of the test compound and an antimicrobial compound of the first class of the antimicrobial compounds.

76. The method of claim 75, wherein the reporter gene is the gene fused to green fluorescent protein.

77. The method of claim 75, wherein the reporter gene is a drug resistance gene

78. The method of claim 75, wherein the reporter gene is detected by immunological screening.

79. The method of claim 75, comprising treating at least two strains of bacterial cells, wherein each of the strains of bacterial cells comprise a responsive promoter linked to a different reporter gene; and detecting the expression of the reporter genes in the bacterial cells; wherein the expression of the at least two reporter genes is indicative of the similarity

or dissimilarity of the mode of actions of the test compound and an antimicrobial compound of the first class of the antimicrobial compounds.

80. The method of claim 79, wherein the strains of bacteria form a set of reporter strains capable of distinguishing the modes of action among two or more classes of antimicrobial compounds.

81. A set of at least two bacterial reporter strains capable of distinguishing the modes of action among two or more classes of antimicrobial compounds, wherein the bacterial strains comprise a responsive promoter linked to a reporter gene; wherein each of the responsive promoters is a promoter which is induced in a cell which is treated by an antimicrobial compound of a first class of antimicrobial compounds, but not by an antimicrobial compound of a second class of antimicrobial compounds, and wherein the presence, absence or change in the amount of the expression of the reporter genes is indicative of mode of action of a test antimicrobial compound.